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Humoral Immune Responses to VP4 and Its Cleavage Products VP5* and VP8* in Infants Vaccinated with Rhesus Rotavirus

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The humoral immune response to rhesus rotavirus (RRV) VP4 and its cleavage products VP5* and VP8* was determined in paired serum samples from 44 infants vaccinated with RRV or human rotavirus-RRV reassortants and 5 placebo recipients. Our aim was to try to measure the response to those regions of VP4 most closely related to protection. An enzyme-linked immunosorbent assay (ELISA) was used to measure the immunoglobulin G immune response to baculovirus-expressed full-length RRV VP4, full-length VP8*, and the amino-terminal polypeptide of VP5* called VP5*(1) (amino acids 248 to 474). The two antigenic regions of VP4 selected for study, VP5*(1) and VP8*, have previously been shown to contain most of the cross-reactive and strain-specific neutralization epitopes, respectively, while the remaining carboxy-terminal half of VP5* (amino acids 475 to 776) has not been clearly associated with neutralization. All three recombinant proteins were antigenically conserved, since they reacted with a library of neutralizing monoclonal antibodies directed at VP4. There was a high percentage of seroresponders to VP4 (61%) or to VP8* (52%), but fewer infants seroresponded to VP5*(1) (11%). In addition, infants responding to VP5*(1) had considerably lower titers than to VP4 or VP8*. Immune response to VP4 correlated strongly with the responses detected by the plaque reduction neutralization assay but did not correlate with the responses detected by the ELISA to whole RRV. These data imply that the VP5*(1) region is less immunogenic than the VP8* region of VP4 in infants immunized with RRV or RRV reassortants. The low immunogenicity of VP5* might adversely affect the efficacy of RRV vaccine candidates.

Rotaviruses have two outer capsid proteins, VP4 and VP7, which are involved in viral neutralization (12, 14, 16, 31, 44). VP7, usually the product of gene 9 (12, 19), is the major surface glycoprotein (23) and is the primary determinant of viral serotype (13, 19, 30). VP4 (86.5 kDa) is the product of gene 4 (1, 18, 27), forms a spike-like structure which protrudes from the viral surface by more than 10 nm (34, 35, 48), and is the viral hemagglutinin (18). VP4 is cleaved by trypsin to VP8* (27 kDa) and VP5* (60 kDa), resulting in an increase in viral infectivity (6). Antibodies to either VP7 or VP4 actively protect pigs against homologous challenge (15). In addition, antibodies to VP7 and to either of the two proteolytic products of VP4 have been shown to passively protect mice against heterologous rotavirus challenge (28, 32). By using monoclonal antibodies (MAbs) in vitro, it has been possible to identify both homotypic and heterotypic neutralization domains on both VP4 and VP7; however, neutralizing antibodies to VP7 tend to be serotype specific (5, 30), while antibodies to the VP5* region of VP4 are generally cross-reactive (27, 43), and antibodies to the VP8* region of VP4 are mostly strain specific (20, 27).

The relative immunogenicities of VP5* and VP8* following infection of humans with human or animal rotavirus strains has not been elucidated. In one study, Svensson et al. were able to detect antibodies to VP5* but not to VP8* by using a radioimmunoprecipitation assay on serum samples from children infected with rotavirus (41). An understanding of the immunogenicities of VP5* and VP8* following infection might improve our ability to predict the degree of heterotypic versus strain-specific immunity that a candidate vaccine may confer.

We addressed the question of the relative immunogenicities of VP4, VP5*, and VP8* by developing an enzyme-linked immunosorbent assay (ELISA) which used three baculovirus-expressed recombinant rhesus rotavirus (RRV) proteins, VP4, VP8*, and an amino-terminal fragment of VP5* named VP5*(1) (amino acids 248 to 475 of VP4), to measure the serum immunoglobulin G (IgG) response in infants vaccinated with RRV or human rotavirus-RRV reassortants.

MATERIALS AND METHODS

Serum specimens. Forty-nine paired serum samples from infants who had participated in phase 1 rotavirus vaccine trials from 1986 to 1989 at the University of Maryland were studied. Thirty-one infants were under 8 months of age, 5 were 8 to 12 months, and 13 were 12 to 22 months old. The serum specimens had been collected 1 day before or on the day of vaccination or 28 days after oral administration of placebo, RRV (serotype G3) vaccine, monovalent human

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rotavirus-RRV reassortant vaccines (D × RRV, serotype G1; DS1 × RRV, serotype G2; or ST3 × RRV, serotype G4), or quadrivalent vaccine consisting of equal amounts of RRV and each of the three reassortants. Each reassortant derived the VP7 gene from the human rotavirus parent and the remaining 10 genes from RRV (29). Monovalent vaccines were given at a dose of 10^4 PFU, and quadrivalent vaccine was given at a dose of 10^4 PFU for each component.

From the more than 200 vaccinees and placebo recipients who participated in these trials, paired serum samples from 30 seroresponders and 14 nonseroresponders in the plaque reduction neutralization (PRN) test and from 5 placebo recipients were included in this study.

Cells and viruses. Wild-type *Autographa californica* nuclear polyhedrosis virus (*Baculoviridae*) or recombinant strains expressing VP4, VP5*(1), or VP8* were grown as described previously (7, 25) in *Spodoptera frugiperda* Sf-9 cells in Excell 400 medium (JR Scientific, Woodland, Calif.).

As a source of antigen for the ELISA, cells infected with wild-type, VP5*(1)-, or VP8*-expressing strains were resuspended in 50 mM Tris-HCl (pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride and sonicated for 30 s at 40 W. Cell supernatant from the VP4-expressing strain was treated with phenylmethylsulfonyl fluoride and used without further treatment. Cell supernatant from the VP4-expressing strain has been found to contain significant amounts of VP4 (25), while VP8* and VP5*(1) are not found in significant amounts in the supernatant of cells infected with the appropriate baculovirus strains (7, 8).

VP4, VP5*(1), and VP8* ELISA. Immulon 2 (Dynatech Laboratories, Inc., McLean, Va.) microtiter plates were coated with a predetermined dilution of antigen in phosphate-buffered saline containing 0.05% sodium azide (PBS-Az). After overnight incubation at 4°C, the plates were washed twice with PBS-Az and blocked with 10% fetal bovine serum in PBS-Az for 12 to 24 h at 4°C (all further washings were done with PBS-Az, and all incubations were done with 5% fetal bovine serum in PBS-Az, unless otherwise indicated). The plates were then washed twice and duplicate wells were incubated with serial twofold dilutions of human serum samples for 2 h at 37°C. After the plates were washed four times, alkaline phosphatase-conjugated goat anti-human IgG (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) was added and incubated for 1 h at 37°C. After the plates were washed four times, the substrate (1 mg of *p*-nitrophenylphosphate per ml in 1 mM $MgCl_2$–1% diethanolamine buffer [pH 9.8]) was added, the plates were incubated for 1 h at 37°C, and the A_{410} was read in an automatic ELISA plate reader (Bio-Tek model EL-310). All serum samples were tested once, and results are expressed as the average A_{410} for duplicate wells.

When the assay was done on plates coated with control antigen (from cells infected with wild-type baculovirus), the mean A_{410} value obtained with the lowest dilution of all pre- and postvaccination sera was 0.079, with a standard deviation of ±0.056 (control standard deviation). The titer against VP4, VP5*(1), or VP8* was defined as the highest dilution of serum giving an A_{410} at least two control standard deviations higher than the value obtained with the same dilution of serum on the control plate. Seroresponse was defined as a titer increase of at least fourfold.

In order to control the relative amounts of the three recombinant proteins used to coat the ELISA plates, two neutralizing MAbs specific for either of the two trypsin cleavage products of VP4 (MAb M2, specific for VP5*, and MAb 7A12, specific for VP8*) (27) were selected from a larger panel of anti-VP4 neutralizing MAbs, such that excess amounts of each MAb produced a similar A_{410} reading (1.0 ± 0.4) when excess amounts of each MAb were used to develop VP4-coated plates. The MAbs selected were considered to have similar avidities, since excess amounts of each MAb produced similar readings when assayed on plates coated with equimolar amounts of VP5* and VP8*, i.e., coated with VP4.

MAbs 7A12 and M2 were then used to select the concentration of the coating antigens VP4, VP5*(1), and VP8* that produced comparable A_{410} readings (1.0 ± 0.4) with their corresponding MAbs when run simultaneously. It was found that the assay was relatively insensitive to antigen dilution, i.e., plateau absorbances characteristic of each batch of the protein of interest were obtained over a wide range of dilutions. This apparent insensitivity to antigen concentration is presumably the result of using nonpurified antigen, in which case the maximum signal obtainable is determined by the ratio of the recombinant to Sf-9 cell-derived proteins. As a result, the concentrations of the coating proteins were indirectly selected by assaying several batches of the proteins of interest (at a single dilution of 1:100) and then selecting those that produced the target A_{410}, rather than directly selecting the concentration of those proteins by testing single batches at different dilutions.

PRN. PRN and IgG ELISA tests with whole, nonpurified RRV were done as described previously (17, 24).

Statistic analysis. McNemar's test for correlated rates (a modified chi-squared test) was used to analyze the differences between the rates of seroresponse as determined by the various methods (36). Since the chi-squared test requires each of the measurements to be independent, and two measurements on a serum sample from the same individual are not independent, McNemar's test rearranges the 2 × 2 contingency table in order not to count twice the measurements obtained for the same individual. In addition, a test of significance based on the coefficient of correlation r (2) was used.

RESULTS

Description of the method. In order to analyze the immune response to RRV VP4 and the two trypsin cleavage products of VP4, we developed an ELISA based on the use of baculovirus-expressed full-length VP4 (776 amino acids), full-length VP8* (247 amino acids), and a fragment of VP5* named VP5*(1), which contains the amino-terminal 227 amino acids of VP5*. Neutralization epitopes on all three recombinant proteins are conserved, since they reacted with a library of neutralizing MAbs and have been demonstrated to be able to induce neutralizing antibodies in parenterally immunized animals (7, 8, 25).

Of the 44 vaccinated infants studied, 36 were given RRV (serotype G3) and 8 were given human rotavirus-RRV reassortants: 2 each received D × RRV (serotype G1), DS1 × RRV (serotype G2), ST3 × RRV (serotype G4), and the quadrivalent vaccine RRV(TV), consisting of equal amounts of RRV and each of the three reassortants. Each of the vaccine formulations contained VP4 derived from RRV. Hence, in this study, only homologous immune responses to RRV VP4 were investigated.

IgG immune response to VP4, VP5*(1), and VP8*. The ELISA titers for IgG to RRV VP4, VP5*(1), and VP8*, ELISA titers for IgG to RRV (whole nonpurified virus), and PRN titers to RRV for 44 vaccinated infants and five placebo recipients are shown in Table 1.

TABLE 1. Immune responses as measureed by IgG ELISA to baculovirus-expressed RRV proteins VP4, VP5*(1), and VP8*; by IgG ELISA to whole-virus RRV; and by PRN of RRV in pre and postinfection serum samples from 44 infants vaccinated with RRV or human rotavirus-RRV reassortants and in five infants who received placebo

Infant no.	Vaccine[a]	Reciprocal IgG ELISA titer[b]: VP4		VP5* (1)		VP8*		IgG ELISA titer[b] to RRV (whole virus)		PRN titer[b] to RRV	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	RRV	–	–	–	–	–	–	1,600	3,200	–	**80**
2	RRV	100	**1,600**	–	–	–	**800**	3,200	3,200	40	**320**
3	RRV	–	**1,600**	–	–	–	**400**	400	**6,400**	–	**320**
4	RRV	100	200	–	–	–	–	6,400	6,400	40	80
5	RRV	100	**400**	–	–	–	–	6,400	3,200	40	**160**
6	RRV	–	**1,600**	–	–	–	**400**	3,200	3,200	–	**1,280**
7	RRV	–	–	–	–	–	–	1,600	3,200	40	40
9	RRV	100	100	–	–	–	–	1,600	**6,400**	40	40
10	RRV	–	**1,600**	–	–	–	**200**	10	**40**	±	**640**
11	RRV	400	400	–	–	200	100	40	**320**	80	80
12	RRV	–	**3,200**	–	**200**	–	**3,200**	–	**40**	±	**2,560**
13	RRV	–	**6,400**	–	–	–	**1,600**	40	**160**	±	**2,560**
14	RRV	–	**3,200**	–	100	–	**1,600**	–	**80**	±	**2,560**
15	RRV	–	**12,800**	–	100	–	**3,200**	20	10	±	**2,560**
16	RRV	–	**400**	–	–	–	100	–	–	±	**640**
17	RRV	–	**3,200**	–	100	–	**1,600**	40	10	±	**2,560**
18	RRV	–	**6,400**	–	**200**	–	**3,200**	–	**40**	±	**2,560**
19	RRV	–	**1,600**	–	–	–	**400**	NT[c]	NT	–	**320**
20	RRV	–	**1,600**	–	–	–	**400**	NT	NT	–	**320**
21	RRV	–	**800**	–	100	–	**200**	NT	NT	–	**1,280**
22	RRV	–	**3,200**	–	**200**	–	**1,600**	NT	NT	–	**1,280**
23	RRV	–	**800**	–	–	–	**400**	NT	NT	–	**80**
24	RRV	–	–	–	–	–	–	1,600	3,200	–	**80**
25	RRV	–	–	–	–	–	–	6,400	3,200	80	**320**
26	RRV	–	–	–	–	–	–	1,600	3,200	40	40
27	RRV	–	–	–	–	–	–	1,600	**6,400**	40	40
28	RRV	200	–	–	–	200	–	100	200	40	80
29	RRV	–	–	–	–	–	–	1,600	1,600	80	80
30	RRV	–	–	–	–	–	–	3,200	100	40	40
31	RRV	–	**400**	–	–	–	–	40	**200**	40	**320**
32	RRV	–	**6,400**	–	**200**	–	**1,600**	1,600	800	40	**1,280**
33	RRV	–	**800**	–	–	–	**200**	800	**3,200**	80	**320**
34	D × RRV	–	–	–	–	–	–	800	400	40	40
35	RRV	–	**1,600**	–	–	–	**800**	200	**3,200**	80	**320**
36	ST3 × RRV	–	**200**	–	–	–	–	200	±	40	40
37	DS1 × RRV	–	**1,600**	–	–	–	**400**	±	**3,200**	40	**320**
38	RRV	100	100	–	–	100	–	400	400	–	**40**
39	RRV(TV)	–	**1,600**	–	–	–	**800**	800	**3,200**	80	**1,280**
40	RRV(TV)	400	**1,600**	–	–	–	**800**	3,200	3,200	80	**320**
41	D × RRV	–	**6,400**	–	**800**	–	**1,600**	3,200	3,200	–	**320**
42	D × RRV	–	**1,600**	–	–	–	**400**	±	50	–	**160**
43	RRV	–	–	–	–	100	–	±	**100**	–	**80**
44	RRV	–	–	–	–	–	–	200	±	–	–
45	DS1 × RRV	100	–	–	–	–	–	±	**100**	–	40
8	Placebo	200	200	–	–	200	100	3,200	3,200	40	40
A	Placebo	100	100	–	–	–	–	NT	NT	80	40
B	Placebo	–	–	–	–	–	–	NT	NT	320	320
C	Placebo	–	–	–	–	–	–	NT	NT	±	±
D	Placebo	–	–	–	–	–	–	NT	NT	±	±

[a] Infants were administered RRV vacine (serotype G3) or human rotavirus-RRV monoreassortants D × RRV (serotype G1), DS1 × RRV (serotype G2), or ST3 × RRV (serotype G4), a quadrivalent mixture with equal amounts of the above [RRV(TV)], or placebo.

[b] – and ±, negative serum samples; IgG ELISA titers to recombinant proteins were <100 (–); IgG ELISA titers to whole RRV were either <10 (–) or <50 (±); and PRN titers to RRN were either <40 (–) or <80 (±). Boldface type indicates fourfold or greater seroresponse.

[c] NT, not tested.

The infants had various levels of antibodies in their prevaccination serum samples, as assayed with the different methods: 32 were positive by ELISA to whole RRV, 24 were positive by PRN to RRV, 11 were positive by ELISA to VP4, and 5 were positive by ELISA to VP8*. Most of the prevaccination antibodies are likely to be of maternal origin, considering the young age of the infants.

Of the 44 vaccinated infants, 30 (68%) seroresponded by PRN and 16 (36%) seroresponded by ELISA to whole RRV, while by ELISA to the recombinant proteins, 27 (61%) infants seroresponded to VP4, 23 (52%) seroresponded to VP8*, and only 5 (11%) seroresponded to VP5*(1). The number of seroresponders to either VP4 or VP8* by ELISA was not significantly different from the number of serore-

sponders by PRN ($P < 0.005$) but was higher than the number of seroresponders to VP5*(1) ($P < 0.005$). Correspondingly, the geometrical mean titers (GMTs) of the postvaccination serum samples to VP4 (GMT = 491) or to VP8* (GMT = 220) were higher than the GMT to VP5*(1) (GMT = 64) by factors of 7.6 and 3.4, respectively.

The coefficients of correlation between the PRN results, considered the gold standard, and those with the other serologic methods were calculated. Extremely good correlation was observed between PRN and ELISA postvaccination titers to VP4 ($r = 0.730$, $P < 0.0005$) and VP8* ($r = 0.854$, $P < 0.0005$). On the other hand, in spite of being barely detectable, the postvaccination titers to VP5*(1) detected by IgG ELISA still had a low level of positive correlation with PRN results, but without statistical significance ($r = 0.195$, $P < 0.05$). This is in sharp contrast with the lack of positive correlation observed between PRN and IgG ELISA to the whole virus particles ($r = -0.358$, $P < 0.025$).

Responses to RRV VP4 were not obviously different between the vaccinees receiving RRV, monoreassortants, or the quadrivalent vaccine.

DISCUSSION

Antibodies to VP4 and VP7 have been shown to neutralize rotavirus in vitro and to mediate protection in animal model systems (5, 14, 15, 28, 32, 44). Unfortunately, it is unclear what the role of these proteins is in mediating protective immunity in the field and in stimulating homotypic and heterotypic immunity (3, 33, 41, 46, 47). It is also unclear what the relative contributions of the two proteolytic fragments of VP4 are in this immunologic equation.

In order to develop tools with which to address questions concerning the relative role of immunity to each of the rotavirus surface proteins in mediating protection, we have developed an assay to measure the specific immune response to individual rotavirus proteins or to fragments of rotavirus proteins containing selected neutralization domains. We have used baculovirus-produced recombinant rotavirus antigens as targets to measure the immune response to full-length VP4 as well as the two proteolytic fragments of VP4, VP5* and VP8*. Prior studies with a library of neutralizing MAbs have demonstrated that most, if not all, of the known VP4 epitopes involved in neutralization are preserved on these baculovirus recombinant rotavirus proteins (7, 8, 25).

Studies of the immune response to specific rotavirus proteins in young children have frequently been complicated by the fact that the virus used as a test antigen is distinct from the virus that actually infected the child (3, 41). This methodologic problem is made more acute when the response to serotypically distinct domains, such as those found on VP4 and VP7, is under study (3, 46, 47). For example, the failure of Svensson et al. to detect responses to serotypically diverse VP8* (41) may be the result of serologic differences between his test strains and the viruses actually infecting the patients under study. To avoid this potential problem, we studied the immune response to recombinant RRV VP4 in infants specifically vaccinated with rhesus rotavirus. This has allowed us to directly measure the homologous response to a specific VP4 molecule. The findings are of additional importance because the RRV VP4 protein is included in both RRV and the reassortant human rotavirus-RRV candidate vaccines currently under study in several countries. However, RRV is a simian virus, and it is highly attenuated in humans. It remains to be seen whether the responses to RRV VP4, VP8*, and VP5* described in this study accurately reflect the responses to VP4 that occur in children infected with virulent or attenuated human rotavirus strains.

The immune response to RRV VP4 was frequent, even when it was administered to infants. Seventy-seven percent of the infants who had any evidence of RRV infection demonstrated a significant response to VP4, and 87% of the infants with detectable PRN responses also seroresponded to recombinant VP4. It is likely that most of the infants we studied were undergoing primary rotavirus infections, since most did not have serum responses to whole rotavirus particles of a magnitude associated with a secondary response, and 70% of the infants were under 8 months of age. The high rate of response to VP4 that we observed is in agreement with previous results obtained by radioimmunoprecipitation (41), competition ELISA (10), and neutralization of reassortant viruses (33, 47), but different from those obtained by Western immunoblots (37) or by neutralization of reassortant viruses (46). These differences can probably be ascribed to the use of heterologous viruses or reassortants that are partially heterologous and to differences in the assay systems used. As suggested by Richardson et al., the Western blot studies might be expected to underestimate VP4 responses (37), while interpretation of neutralization assays with various reassortants might be complicated by unexpected interactions between VP4 and VP7.

Using a MAb directed at an immunodominant neutralization epitope on VP5*, Shaw et al. determined that 10 of 24 (41.7%) children vaccinated with RRV developed antibodies that competed with the monoclonal for binding to the virus (38), and Taniguchi et al., by using the same method, found a similar rate of response (10 of 19 [52.6%]) to heterotypic neutralization epitopes on VP5* in children either naturally infected with rotavirus or vaccinated with RRV (45); however, the heterotypic responses observed by Taniguchi et al. occurred only when children possessed antibody to any serotype of rotavirus in their acute-phase or prevaccination serum samples. Svensson et al. also detected frequent seroresponses to VP5* by radioimmunoprecipitation (41). In the current study, we observed that fewer (11%) vaccinees (most of them presumably undergoing primary infections) developed antibodies to VP5*(1). Since the VP5*(1) was truncated and was lacking the carboxy half of the VP5* molecule, these differences may be due to the failure of our assay to measure antibody to all regions of the VP5* molecule. Since the carboxy terminus of VP5* has not been associated with neutralization, immune response to this region may not be significant, however. It is also likely that the patient population studied by Shaw et al. and Svensson et al. might have included more children with secondary responses in whom there was greater response to VP5*. Alternatively, it seems possible, but less likely, that the competition ELISA and the immunoprecipitation assay were more sensitive than the recombinant-protein-based ELISA. Comparison of the sensitivity of our assay and that of the competition ELISA is complicated, however, by the possible contribution to the latter of antibodies directed to nonrelated epitopes on the same or even on different proteins. The direct ELISA has the advantage of being very simple to run in large numbers and being able to measure responses to a whole protein rather than a specific epitope. The relative utility of the competition assay and the direct assay for evaluating the immunity of individuals will need to be studied directly.

The response to RRV measured by PRN correlated extremely well with serum IgG response to VP4 or VP8* but

did not correlate with the response to whole virus as detected by ELISA. The neutralization assay measures a combination of responses to VP4 and VP7, while the whole-virus ELISA primarily measures the response to VP6, as indicated by the finding that most hybridomas obtained after immunizing mice with double-shelled rotavirus and screened by ELISA or solid-phase radioimmunoassay as the primary screening technique have been observed to be directed to VP6 (11, 40), whereas a small number have been observed to be neutralizing (4, 22). It remains to be determined how useful the VP4 ELISA will be as a surrogate assay to study the neutralization response to RRV vaccination in other field studies, but this initial study appears promising.

In this study, we did not measure the response to VP7. Studies from several laboratories have indicated that the primary neutralization domain of VP7 is composed of several distinct and nonlinear coding sequences from at least two parts of the molecule (26, 39, 42). To date, it is unclear whether this conformation-dependent VP7 neutralization domain is present on any recombinant VP7 protein. An ELISA designed to measure the immune response to VP7 would be most useful if the VP7 recombinant antigen contained the major VP7 neutralization epitope. Studies to measure the response to VP7 by using recombinant protein are under way in our laboratory.

We were surprised to find that the frequency of response to VP8* was about the same as the response to full-length VP4 but that the immune response to VP5*(1) was substantially less frequent. This is not likely to be due to poor preservation of the antigenicity of the recombinant VP5*(1), since this construct reacts with several neutralizing MAbs and has been shown to be able to induce neutralizing antibodies in immunized animals (8). We specifically designed our assay to include only that portion of VP5* previously linked to neutralization epitopes in order to optimize the correlation of ELISA response with neutralization response. While it is possible that use of a full-length VP5* molecule might have detected more seroresponses, the antigens found on the carboxy-terminal half of VP5* have not been clearly associated with neutralization (9, 27). Our aim was to try to measure the response to the regions of VP4 most closely related to protection.

It has been demonstrated by several groups that the epitopes on VP8* are mostly strain specific, while those on VP5* are mostly cross-reactive (21, 27, 43). Our findings indicate that infection with viruses containing RRV VP4 primarily stimulates an immune response to VP8*. Unfortunately, an immune response to RRV VP8* seems highly unlikely to induce protective immunity to human rotavirus strains. Taniguchi et al. recently suggested that cross-reactive neutralization epitopes on VP5* are weakly immunogenic after either vaccination or natural primary rotavirus infections (45). A low-level immune response to VP5* might be improved by primary vaccination with recombinant VP5* or VP4. Since neutralizing antibodies recognizing VP5* are frequently cross-reactive, immunization with VP5* might be expected to lead to a more cross-reactive immune response.

We anticipate that immunoassays with recombinant human rotavirus VP4 or other rotavirus proteins could be used to study the specificity and magnitude of the immune response to individual rotavirus proteins in infants naturally infected with rotavirus. In such an assay, the use of recombinant proteins from a strain homologous to the infecting virus would be most revealing. It will be important to determine whether the immune response to human rotavirus infection is quantitatively and qualitatively similar to the response to heterologous infection with RRV. The extended replication of the homologous virus might enhance VP5*-directed immunity and hence promote a more heterotypic response.

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